ER-00M-000

Outline guidance for QA/QC

This QA/QC guidance is for【Validation study of the LUMI-CELL® Assay for screening of endocrine disrupter】 specifies action necessary to maintain quality accuracy and quality control of the laboratory.

I. Quality Control System

1. Organization

   Appropriate management of quality control system is secured for 【Validation study of the LUMI-CELL® Assay for screening of endocrine disrupter】with the following personnel;

   Mr. Hiroshi Murata – Facility Management
   Mr. Masafumi Nakamura – Study Director/Safety Officer
   Dr. Hajime Kojima – Director of Quality Assurance
   Mr. Tsukasa Yamamoto – Consultant
   Mr. Hiroshi Handa – Laboratory Technician

2. Documentation

   Any document and record stipulated in this guideline shall be submitted in original to the Facility Management and a copy to the Director of Quality Assurance with the date of creation written.

3. Standard Operating Procedure

   Study Director needs to prepare “LUMI-CELL®ER International Validation SOW” and “LUMICELL ER Assay”(Japanese Version) and submit to Facility Management and Director of Quality Assurance.

4. Implementation Plan

   Study Director should make “LUMICELL®ER validation implementation plan” for each phases and submit to Facility Management and Director of Quality Assurance.

   - “LUMICELL®ER validation implementation plan” should include items (method, protocol, research place, etc), implementation term, draft implementation plan, schedule plan.

5. Final action table

   Laboratory Technician shall record daily work and change of plans in 【Validation study of the LUMI-CELL® Assay for screening of endocrine disrupter daily report】. Date, Work descriptions, number, work time, special instruction, signature by work person and supervisor should be included.

   Work description are: 1) Sample acceptance 2) Adjustment (prepare standard and

provided sample) 3) Cell (thaw, subculture, store, preparation of media) 4) Assay (seeding, determine dilution rate, responsive reaction, Luciferase assay, toxicity assay) 5) Data analysis.

II. Management of chemicals, supplies, devices, and facilities

1. Chemicals

a) Laboratory Technician shall handle solvent according to "Chemical Adjusting Standard Operating Procedure (ER-80S-120)

- Date, name of work person, chemical lot number, used media number, and bottle number shall be recorded in the "Used Chemical Log book" when chemicals are used. Term of expiration for using chemicals is 3 months but if there is any problem when using it, all chemicals used shall be discarded at the same time.

- Media number shall be recorded to "Cell recovery, subculture, and seeding procedure record book" when using it.

- When disposing used chemicals, label on bottle or box shall be stored for history record.

- Periodically weigh remaining chemical volume (once a week)

2. Standard solutions and samples

a) Laboratory Technician shall handle solvent according to "Sample Adjustment Operating Procedure"(ER-80S-110)

- record date, work person's name with sign, supervisor's name and sign, standard solution weigh, amount of DMSO, dissolving procedure (1)-(6), method of storage, adjustment shall be recorded when adjusting standard solution. Control number, name of standard solution, adjusted concentration, adjusted date, term of expiration (one year after date of adjusting) shall be recorded to "Standard Curve, Control Solution Use Record" for information when used.
- Record date, work person's name, volume, amount of DMSO, concentration, and dissolving method(1)-(6) in the remarks column of the "Sample Record Book" after the samples are used.
- Record date, user's name, used amount, remaining amount, and test description in the remarks column of "Standard Curve, Control Solution Used Record" when using standard solutions.
- Record date, user's name, used amount, remaining amount, and description in remarks column of "Sample Record Book" when using samples.
- Write down the used dissolving method, by selecting one of the below mentioned methods.
  1) Shaked with hand for 30 second

2) Dissolved using voltex for 30 second
3) Dissolved using ultrasonication for 30 second
4) Dissolved by heat (37C) for 5 minutes
5) Dissolved using ultrasonication for 30 second
6) Dissolved using voltex for 30 second

- Sample Storage Method:

  Room temperature: Standard solution and samples stored in lockable storage place.

  Cool temperature: Standard solution and samples stored in lockable storage place.

  Freezing: Standard solution and samples stored in lockable storage place.
- Periodically weigh remaining chemical volume (once a week)
- For DMSO after opening a new 100 ml bottle of DMSO, add 2 ml to 4ml vial, seal with a Teflon seal and write down the stored date on each vial. First check the quality of DMSO. If it has been used for more than one month then dispose it and open a new vial of DMSO.

3. Equipment

a) Laboratory Technician shall handle micro pipette according to "Micropipette Operating Manual"(ER-80A-05)

Check items in "Equipment Checking List" once a month. Confirm performance once half-year (September and March). Check items in "Facility Daily Checking List" every time before using it.

4. Device

a) Laboratory Technician shall handle electronic analytical balance according to "Electronic Analytical Balance Operating Manual)" (ER-80A-01) when weighing standard solution and samples.

Check items in "Equipment Checking List" once in a month. Conduct regular inspection once a year and create work report. Check items in "Equipment Daily Checking List" every time before using it.

If the balance has printing option, printout data and attach it to "Standard Solution Record Book" for standard solution and "Sample Record Book" for samples.

b) Laboratory Technician shall handle CO2 Incubator for cell culture according to "CO2 INCUBATOR OPERATING MANUAL".

Check items in "Equipment Checking List" once in a month. Conduct regular inspection once a year and create work report. Check items in "Equipment Daily Checking List" every time before using it.

Cleaning: Clean inside the incubator once in two months (even month)

Change of humidifying water: Change humidifying water once in two weeks.

Connection of Co2 compressed gas cylinder: Confirm remaining CO2 volume every time before using.

Temperature: Temperature shall be checked with attached digital thermometer and recorded. Once a week, temperature shall be checked with standard thermometer and recorded.

c) Laboratory Technician shall handle autoclave for sterilizing according to "Autoclave Operating Manual" (ER-80A-03).

Check items in "Equipment Checking List" once in a month. Also conduct inspection once a year based on occupational safety regulation and store the result for three years. Check items in "Facility Daily Checking List" every time before use.

d) Laboratory Technician shall handle safety cabinet and fume hood for sterilizing procedure according to " Safety Cabinet and Clean bench Operating Manual".

Monthly check items in "Equipment Checking List". Check items in "Facility Daily Checking List" every time before use.

e) Laboratory Technician shall handle luminometer according to "Luminometer Operating Manual" (ER-80A-06).

Monthly check items in "Equipment Checking List". Conduct regular inspection once a year and create work report. Check items in "Facility Daily Checking List" every time before use.

Before using, use test plate and record RLU and room temperature and when finished using, record the number of plate analyzed.

On regular inspection, calibrate using luciferase standard and record the result.

f) Laboratory Technician shall handle room temperature storage, refrigerator, freezer for chemicals and sample storage according to "Room temperature Storage, Refrigerator, and Freezer Operating Manual"(ER-80A-07).

For the Refrigerator and freezer, monthly check items in "Equipment Checking List". Check items in "Facility Daily Checking List" every time before use.

Cleaning: Conduct refrigerator and freezer cleaning once in two months (even month)

Temperature check: Daily temperature check shall be done using attached digital thermometer and weekly check shall be done once a week using standard thermometer and record the data.

5. Facility

a) Specification: The facility shall be designed in double door to prevent people and outside air to directly come inside the room. High concentration and low concentration samples are treated in different room for pretreatment process. The room should have effluent gas and water treatment facility to prevent leaking of hazardous substances.

b) Management of entrance and exit: Restrict entrance only to authorized personnel and other entrance shall be recorded by “Entrance/Exit record” （DKA-80A-V02）

c) Room environment

1) Room temperature: within 20-25C
2) Moisture: within 30-70%
3) Room pressure: negative pressure
4) Intake air filter: No clogging or adsorption breakthrough
5) Disposing air filter: No clogging or adsorption breakthrough
6) Activated carbon for effluent water treatment: No clogging or adsorption breakthrough

d) Room for cell treatment

Sample will be measured using cell and determine dioxin quantity. Storage and subculture is also done in the room.

1) The room is designed in double door to prevent people and outside air to directly come inside the room.
2) Install safety cabinet and fume hood for work using cell
3) Use HEPA filter for air intake and emission for safety cabinet.
4) Control room temperature and moisture using air conditioner (20-25C, temperature change within 3C/day, 30-70%)
5) Maintain room under negative pressure by fume hood or other air evacuation (Room differential pressure 2-4mmH2O).
6) Attach HEPA filter and activated carbon filter for air intake.

III. D

1. Check on sample receipt

Accept samples for Lumi-cell ER assay according to “Sample Acceptance Operating Procedure”(ER-15M-100).

- After receiving the samples, record date, product name, manufacture, lot no., concentration, package, purchase amount, date opened, term of expiration, control number, and means of storage to “Standard Solution Purchase Record”. Also, record sample name, sample number, received date, received method, received amount, received condition, and means of storage to “Sample Record Book” for information when using it.

- When received standard solution, record date, product name, manufacture, lot no., concentration, package, purchase amount, date opened, term of expiration, control number, and means of storage to “Standard Solution Purchase Record”. Also record control number, standard name, manufacture, and purchased date to “Sample Record Book” for information when using it.

2. Preparation of the cell

Handle the cell line according to “Cell Operating Procedure”(ER-60M-100)”

- When sub culturing, collecting, or substituting to DMEM media, record dilution rate, number of times sub cultured, number of flask, used media number, contamination status, working time, and name of work person for every lot cell
- When thawing cell, record date and name of work person for every control number to “Cell control book” and record used cell number to “Cell recovery, subculture, and seeding procedure record book”
- When storing cell, record date, name of work person, canister number, cane number, cell concentration, origin of storing cell, and amount stored to “Cell control book”

3. Measuring sample

Measure samples according to “Assay Operating Procedure”(ER-60M-110)

- When seeding, record number of times sub cultured, number of flask, used media number and cell condition (growth speed, media condition, checking with microscope) for each cell lot and record number (date-work person’s initial-No) to the seeded plate.
- Record plate layout, media number, plate number, standard curve number, QC solution number, work date, working person, dosing number (date-working person’s initial-No) for Agonist test (determination of dilution rate, response reaction) and antagonist test (determination of dilution rate, response reaction).
- When measuring luciferase activity, record cell viability score (refer to “LUMI-CELL®ER ASSAY Visual Observation Cell Viability Manual” for score), lysing solution number, substrate number, date, work person’s name to “Dosing, Lysing, and Assay Operating Procedure”

IV. Confirmation and Reporting of Result

1. Result analysis

- Save as excel data and printout output data from luminometer. Analyze the data using following files for each test:
  1) Agonist test (determination of dilution rate); [AgRF]
  2) Agonist test (response reaction); [AgComp]
  3) Antagonist test (determination of dilution rate); [AntRF]
  4) Antagonist test (response reaction); [AntComp]
- Laboratory Technician shall record Plate #, DMSO Lot #, Media Lot # and Standard, QC, Compound Name and Hiyoshi Control ID# to [Compound Tracking Form] on each sheet and paste data from luminometer to [RAW DATA]. Record compound concentration, date, work person’s name to [LIST] and [REPORT] and submit to Study Director/Safety Officer.
- Study Director/Safety shall record Pass/Fail based on data acceptable criteria, and date, and worker’s name and submit electric file to Facility Management and International Study Management Team.

2. Reporting result

- Creating Historical Database

Study Director/Safety shall printout obtained result and accumulate Historical Database to each test file;

1) Agonist test: [LUMIAgonistQC]

2) Antagonist test: [LULMIAntagonist QC]

- Data acceptable criteria

Data acceptable criteria is made based on Phase I result. This criteria will be used for determination of result. Result will determine as fail if it does not fit within the criteria.

- QC Scatter Charts (Agonist)

1) Methoxychlor Control criteria: average±2.5SD

2) E2 EC50 criteria: average±2.5SD

3) DMSO Control criteria: average±2.5SD

4) Assay Induction criteria: >Fold Induction[3]

- QC Scatter Charts (Antagonist)

1) Flavone Control criteria: average±2.5SD

2) Raloxifene/E2 IC50 criteria: average±2.5SD

3) DMSO Control criteria: average±2.5SD

4) Assay Reduction criteria: >Fold Induction[3]

3. Storage and submission of result

a) Storage and submission of LUMI-CELL Data

Study Director/Safety shall gather [Compound Tracking Form], "Dosing, Lysing, and Assay Operating Procedure", and "Luminometer Raw Data print-out sheet" and save original and one copy as hard copy, file in "LUMICELL ER® RAW DATABASE" for each Phase and at the end of each phase, submit original to Facility Management and one copy to Director of Quality Assurance.

b) Storage and submission of LUMI-CELL QA, QS

Study Director/Safety shall copy all recorded document regard to QA/QS and file it to "LUMICELL ER® RAW DATABASE" by each Phase and submit original to Facility Management and one copy to Director of Quality Assurance.

Remarks:

【 】: Title " ": Document and record

[ ]: Electric file ( ): Complement